# THE EFFECTS OF SULFANILAMIDE UPON THE OXYGEN UPTAKE OF MITOTICALLY ACTIVE AND BLOCKED GRASSHOPPER EMBRYOS[1]

JOSEPH HALL BODINE

*Zoological Laboratories, State University of Iowa, Iowa City, Iowa*

The effects of sulfonamides upon bacteria have been repeatedly pointed out and various ideas as to their fundamental action in such cases have been advanced (Fildes, 1940; Woods and Fildes, 1940; Woods, 1940; Eyster, 1943; Steers and Sevag, 1949). That sulfonamides markedly interfere with such general cell activities as division and growth seems also well established (Fisher, Henry and Low, 1944). More specific effects of these reagents upon cellular enzyme systems have also been investigated and rather striking concepts of their modes of action demonstrated (Johnson and Chase, 1942; Mann and Keilin, 1940; Johnson, Eyring and Williams, 1942). Marked inhibitions of cell division, oxygen consumption and enzyme activity have been reported and of especial interest are the relations found between the effects of sulfanilamide upon oxygen uptake and cell division in various types of animal and plant cells (Fisher, Henry and Low, 1944). Fisher, Henry and Low (1944), for the sea urchin egg, have shown that when the cell division is stopped by sulfanilamide, the oxygen uptake and especially the activity system is also completely inhibited. In this respect the action of sulfanilamide seems to resemble that of certain narcotics (Johnson, 1942).

Since the embryo of the grasshopper in its development at constant temperature (25° C.) normally goes through a block or diapause period in which mitotic activity is completely stopped it becomes of some interest to determine the action of sulfanilamide on it during both the blocked and active phases of its development. Morphologically similar embryos in mitotically active and blocked states are readily available and lend themselves to such an investigation (Bodine and Boell, 1936). The present paper, therefore, deals with data concerning the effect of sulfanilamide upon the oxygen uptake of morphologically identical grasshopper embryos both in mitotically active and blocked conditions.

## Material and Methods

Embryos, free from yolk, were obtained from eggs of the grasshopper, *Melanoplus differentialis*, as previously noted (Bodine and Boell, 1936). Oxygen determinations were carried out at 25° C. with Warburg manometers, using respiration flasks of 5 ml. capacity. Embryos were suspended in phosphate buffered (pH 6.8) NaCl solution (0.65 per cent) and solutions of sulfanilamide were also made up

[1] Aided by grant from the National Institutes of Health. Acknowledgment is gratefully made to L. R. Fitzgerald, Etta Andrews, John Johnston and Herman Tharp for technical assistance in carrying out these experiments.

in it. Further details of procedure have already been described (Bodine and Fitzgerald, 1949).

## Results

The effects of sulfanilamide, ranging in concentration from 0.005 M. to 0.06 M., on the oxygen uptake of both active and blocked embryos were determined. Results of many such experiments are graphically shown in Figure 1. An inspection of this figure shows that sulfanilamide depresses the rate of oxygen uptake of both active and blocked cells to approximately 50 per cent of the normal value. No

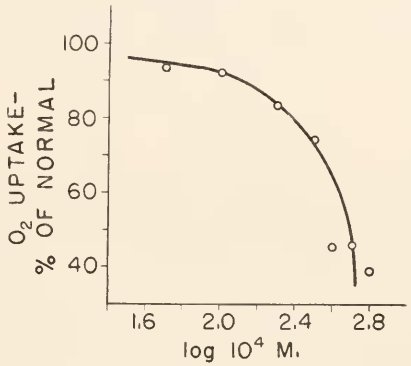


Figure 1. Shows the effect of sulfanilamide on the oxygen uptake of active and blocked embryos. Abscissa, log $10^4$ M. sulfanilamide; ordinate, relative oxygen uptake rate, taking $O_2$ uptake of non-treated embryos as 100%. Circles represent averages of all experiments.

significant differences in responses between the mitotically active and blocked cells have been found (Table I). The effects of sulfanilamide upon the oxygen uptake do not seem to be conditioned by the mitotic activity of the cells of the embryos. By choosing embryos in various degrees of diapause or cellular block (as shown by rate of oxygen uptake) it is possible to show that the relative effects of sulfanilamide are similar in all cases. In Figure 2 are graphically shown the results of several experiments using 0.06 M. sulfanilamide and embryos in different levels of block. The degree of block or diapause in these embryos is indicated by their rates of oxygen uptake, i.e., embryos in complete block have much lower rates of oxygen consumption than similar ones out of block and actively developing (Bodine and

Table I

*Inhibition of $O_2$ uptake in % of normal for embryos*

| Diapause | Postdiapause |
|---|---|
| 44 | 70 |
| 48 | 55 |
| 69 | 58 |
| 68 | 74 |
| 65 | 43 |
| 49 | |
| 48 | Av. = 60.0 |
| 56 | |
| Av. = 57.2 | |

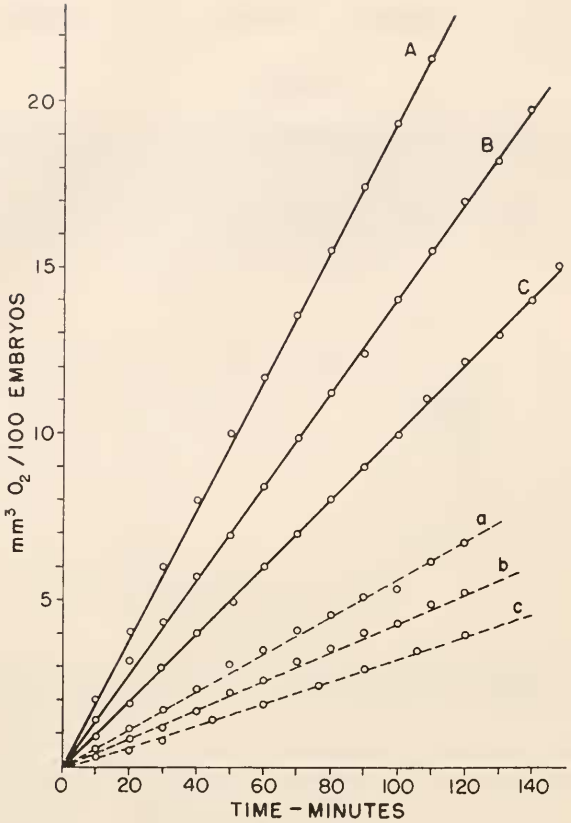


FIGURE 2. Shows effect of 0.06 M. sulfanilamide on the oxygen uptake of morphologically similar diapause and postdiapause embryos. Abscissa, time in minutes; Ordinate, mm.$^3$ $O_2$/100 embryos. A = postdiapause embryos (2 days at 25° C.) in Ringer. a = same embryos exposed to sulfanilamide. B = embryos coming out of diapause. b = same exposed to drug. C = embryos in complete block or diapause. c = same exposed to drug.

Boell, 1936). An inspection of Figure 2 shows that for two-hour exposures to sulfanilamide the relative effects of the drug on the oxygen uptake of these embryos are practically constant throughout. It seems, therefore, that for the grasshopper embryo the particular states of their cellular activity have little to do with their response to sulfanilamide especially as regards their oxygen uptake. It is of some interest to note that the relative amounts of sulfanilamide sensitive (approx. 60–70 per cent) and sulfanilamide insensitive (approx. 30–40 per cent) fractions of the respiration seem to be practically identical in the mitotically active and blocked cells. The inhibitory effects of sulfanilamide for both types of embryos, except for prolonged exposures, are quite reversible.

## Discussion

The above results for the grasshopper embryo appear quite different from those found for the fertilized and unfertilized sea urchin egg (Fisher, Henry and Low, 1944). Fertilized sea urchin eggs behave quite like the grasshopper embryo while

unfertilized eggs seem to be quite resistant to the action of sulfanilamide. The relative degrees of inhibition of the oxygen uptake for the fertilized sea urchin egg and the grasshopper embryo are quite similar (55–60 per cent). No cytological examinations of sulfanilamide treated active grasshopper embryos have been made to determine possible cellular effects and especially as regards effects on cell division. However, the blocked grasshopper embryo contains no mitotically active cells when in true diapause (Slifer, 1932) and its respiratory rate perhaps represents a real basal one. To what extent one is justified in comparing the physiological differences in response to sulfanilamide of the sea urchin egg and the embryonic cells of the grasshopper is quite an open question. For the grasshopper embryo one may reasonably conclude, however, that the inhibitory effects of sulfanilamide on the oxygen uptake seem to be quite independent of the mitotic behavior of the embryonic cells.

## Summary

1. The effects of sulfanilamide upon the oxygen uptake of mitotically active and blocked grasshopper embryos have been determined.

2. Sulfanilamide depresses the rate of oxygen consumption in both active and blocked embryos in a relatively similar fashion.

3. The sulfanilamide sensitive and insensitive fractions of the respiration are approximately constant for both active and blocked embryonic cells.

## LITERATURE CITED

Bodine, J. H. and E. J. Boell, 1936. Respiration of the embryo versus egg (Orthoptera). *J. Cell. and Comp. Physiol.*, **8**: 357–366.

Bodine, J. H. and L. R. Fitzgerald, 1949. The effects of methylene blue and urethane (ethyl carbamate) upon the oxygen uptake of embryonic cells. *Physiol. Zool.*, **22**: 283–294.

Eyster, H. C., 1943. Mechanism of sulfanilamide action and its interaction with p-amino benzoic acid. *J. Cell. and Comp. Physiol.*, **21**: 191–197.

Fildes, P., 1940. A rational approach to research in chemotherapy. *Lancet*, **238**: 955–956.

Fisher, K. C., R. J. Henry and E. Low, 1944. The effects of sulfanilamide and azide on oxygen consumption and cell division in the egg of the sea urchin, *Arbacia punctulata*. *J. Pharm. and Exp. Therap.*, **81**: 58–66.

Johnson, F. H., 1942. Mechanism of p-aminobenzoic acid action and the parallel effects of ethyl carbamate (urethane). *Science*, **95**: 104–105.

Johnson, F. H. and A. M. Chase, 1942. The sulfonamide and urethane inhibition of cypridina luminescence in vitro. *J. Cell. and Comp. Physiol.*, **20**: 151–161.

Johnson, F. H., H. Eyring and R. W. Williams, 1942. The nature of enzyme inhibitions in bacterial luminescence: sulfanilamide, urethane, temperature and pressure. *J. Cell. and Comp. Physiol.*, **20**: 247–268.

Mann, T. and D. Keilin, 1940. Sulphanilamide as a specific inhibitor of carbonic anhydrase. *Nature*, **146**: 164.

Slifer, E. H., 1932. Insect development. IV. External morphology of grasshopper embryos of known age and with a known temperature history. *J. Morph.*, **53**: 1–9.

Steers, E. and M. G. Sevag, 1949. The mechanism of resistance to sulfonamides. IV. A comparative study of the amino acid metabolism of *Staphylococcus aureus* in relation to the mechanism of resistance. *Arch. Biochem.*, **20**: 129–143.

Woods, D. D., 1940. The relation of p-aminobenzoic acid to the mechanism of the action of sulphanilamide. *Brit. J. Exp. Path.*, **21**: 74–90.

Woods, D. D. and P. Fildes, 1940. The anti-sulphanilamide activity (in vitro) of p-aminobenzoic acid and related compounds. *Chem. and Industry*, **59**: 133.